A High-Throughput Screening Method to Identify Potential Pesticides for Mosquito Control

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ABSTRACT Mosquitoes that transmit human diseases are of major importance to the international public health community. Pesticides remain a major component of integrated programs to control these medically important species. However, very few types of pesticides are currently registered for mosquito control. A high-throughput screening method using first-instar larvae of *Aedes aegypti* was created and evaluated in our laboratory to quickly screen large numbers of chemicals for activity against mosquitoes. LC_{50} values of a representative group of compounds were determined using this high-throughput screening method and compared with LD_{50} values determined by topical application against female adults of *Ae. aegypti*. Our results show that this high-throughput screening method is suitable for screening large numbers of candidate chemicals quickly to identify effective compounds.

KEY WORDS high-throughput screen, pesticides, mosquito control

Mosquitoes are important to the international public health community because they transmit many disease pathogens. For example, the mosquito *Aedes aegypti* L. (Diptera: Culicidae) transmits viral pathogens of humans, including yellow fever (Gillett and Ross 1955, Philip 1962, Soper 1967, Aitken et al. 1977) and dengue (Mattingly 1967, Rudnick 1967, Coleman and McLean 1973, Degallier et al. 1988), both of which can cause severe human morbidity and mortality. *Culex quinquefasciatus* Say (Diptera: Culicidae) is the vector of the filarial parasite *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae), which causes Bancroftian filariasis in humans (Sabatinelli et al. 1994, Samuel et al. 2004). *Cx. quinquefasciatus* is also a vector of West Nile virus (Godsey et al. 2005), Japanese encephalitis virus (Nitatpattana et al. 2005), and St. Louis encephalitis virus (Jones et al. 2002).

Use of pesticides for mosquito control has been shown to be very effective and safe when used as part of an integrated pest management (IPM) strategy; however, only a limited number of pesticides are currently registered for mosquito control. Furthermore, many mosquito species have developed resistance to various classes of pesticides (Su and Mulla 2004, Tia et al. 2006, Xu et al. 2006). In combination, these two situations create an urgent need to screen large numbers of chemicals to identify potential new effective pesticides that could be used to control these important disease vectors. To determine the activity of a chemical against mosquitoes, a topical application procedure using female *Ae. aegypti* (Pridgeon et al. 2007) has recently been shown to be very accurate and effective. However, to efficiently screen hundreds or thousands of chemicals in a short period of time, limitations on manpower and the ability to produce large numbers of adult mosquitoes become significant. To save time, labor, and money, we decided to evaluate a high-throughput method using freshly hatched first-instar larvae of *Ae. aegypti*. To evaluate the effectiveness of this method, we selectively chose 19 chemicals with known activities against female *Ae. aegypti* and determined their LC_{50} values against the first-instar larvae of *Ae. aegypti*. The results from both methods were compared and analyzed. Our results showed that this high-throughput screening method using first-instar larvae of *Ae. aegypti* is suitable for screening thousands of chemicals quickly to identify effective candidate pesticides.

Materials and Methods

Mosquitoes. All mosquitoes were reared in the insectary of the Mosquito and Fly Research Unit at Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), USDA–ARS. The *Ae. aegypti* colony has been maintained in the insectary since its establishment in1952 from strains collected in Orlando, FL. First-instar larvae were used in all larval assays, and 5- to 7-d-old females were used for all adult assays. Mosquitoes were reared using standard procedures (Reinert et al. 1997, McCall and Eaton 2001, Pridgeon et al. 2007). Briefly, eggs were hatched under

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Table 1. Modes of action of the 19 selected pesticides used in the study

Pesticide name	Modes of action	Group[a]
Bifenzate	Neuronal inhibitors, unknown mode of action	25
Dicofol	Unknown	Unknown
Amitraz	Octopaminergic agonists	19
Propargite	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12C
Hydramethylnon	Mitochondrial complex III electron transport inhibitors	20
Cyhexatin	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12B
Diafenthiuron	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12A
DNOC	Uncouplers of oxidative phosphorylation via disruption of H^+ gradient	13
Azocyclotin	Inhibitors of oxidative phosphorylation, disruptors of ATP formation	12B
Pyridaben	Mitochondrial complex I electron transport inhibitors	21
Chlorfenapyr	Uncouplers of oxidative phosphorylation via disruption of H^+ gradient	13
Indoxacarb	Voltage Dependent Sodium channel blockers	22
Carbaryl	Acetylcholinesterase inhibitors (carbamates)	1A
Spinosad	Nicotinic acetylcholine receptor agonists	5
Imidacloprid	Nicotinic acetylcholine receptor agonist/antagonists	4
Diazinon	Acetylcholinesterase inhibitors (organophosphates)	1B
Abamectin	Chloride channel activators	6
Permethrin	Sodium channel modulators	3
Fipronil	GABA-gated chloride channel antagonists	2

[a] Group according to modes of action × the IRAC.

vacuum (≈1 h) by placing a square of a paper towel with eggs in a flask filled with distilled water containing larval diet (3:2 brewer's yeast:liver powder; MP Biomedicals, Irvine, CA). Larvae were removed from vacuum and held overnight in the flask after which larvae were transferred to a plastic tray containing distilled water. Larval diet was added to each tray, and mosquitoes were reared in an environmental chamber with a temperature profile simulating a summer day regimen (ranging from 22 to 30°C) and 80% RH. Incandescent lighting was set to a crepuscular profile with a photoperiod of 14:10 (L:D) h, including 2 h of simulated dawn and 2 h of simulated dusk. Adults were held in a screened cage and were provided 10% sucrose ad libitum. Bovine blood with 1% heparin placed in a pig intestine and warmed to 37°C was provided to adults twice a week. Eggs were collected on paper towels (Vasco Brands, Elmira, NY) that lined the rim of water containers. These egg-laden papers were air dried at 27°C and 80% RH for 24 h and stored in containers with 100% humidity for 3–30 d. When needed, eggs were hatched under vacuum, and larvae were reared as described above.

Pesticides and Test Chemicals. All registered pesticides were purchased in technical grade from Chem Service (West Chester, PA). Serial dilutions of all pesticides were prepared in acetone. Nineteen pesticides, each representing a different category of pesticide (Table 1), were chosen from the Insecticide Resistance Action Committee (IRAC) Mode of Action (MoA) classification list (http://www.irac-online.org/documents/IRAC%20MoA%20Classification%20v5_3.pdf) and evaluated against the first-instar larvae and female adults of *Ae. aegypti.* All experimental compounds were provided either by the Department of Chemistry, University of Florida, or the Natural Product Utilization Research Unit, USDA–ARS.

Adult Bioassays. Adult bioassays were performed using a topical application method (Pridgeon et al. 2007). Briefly, 5- to 7-d-old females were anesthetized for 30 s with carbon dioxide and placed on a 4°C chill table (BioQuip Products, Rancho Dominguez, CA). A droplet of 0.5 μl of pesticide solution in acetone was applied to the dorsal thorax using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV). Six concentrations of each pesticide, providing a range of 0–100% mortality, were used on 25–30 females per concentration. Tests were replicated three times. Control treatments with 0.5 μl of acetone alone which consistently gave control mortality rates of <10%. After topical treatment, mosquitoes were kept in plastic cups and supplied with 10% sucrose solution for 24 h before mortality was recorded. Temperature and relative humidity were maintained at 26°C and 80% RH, respectively. Every bioassay was conducted at 27°C and 80% RH and replicated three times.

High-throughput Screening Larvae Assays. The high-throughput screening (HTS) larval assays were created as follows. Briefly, five first-instar larvae (24 h after hatch) of *Ae. aegypti* were added to each well of 24-well plates. Deionized water (950 μl) and larval diet (40 μl) were added to each well. All chemicals to be evaluated were diluted in acetone. Decreasing concentrations were used to further group the chemicals as highly active, moderately active, slightly active, or highly inactive. Diluted chemicals (10 μl) were added to each well containing the larvae plus a total volume of 1 ml of food and water. As control treatments, 10 μl of acetone alone was added to each well. Larval mortality was recorded after 24 h of exposure. Larvae that showed no movement in the well after manual disturbance of water and food by a pipette tip were scored as dead. To accurately compare the larval assay method to the topical application method, the LC_{50}/LC_{95} values of the 19 pesticides in this larvae assay were determined using different concentrations of pesticides. For each pesticide concentration, two replicates were used in each larval assay. The larval assays were repeated several times on different days with six concentrations providing a range of 0–100% mortality.

Table 2. Bioassay results of HTS using 19 pesticides against first-instar larvae of *Ae. aegypti*

Pesticide	Dead/total				
	2,000 ppb[a]	500 ppb[a]	31.25 ppb[a]	2 ppb[a]	0.5 ppb[a]
Bifenzate[b]	5/5	0/5	0/5	0/5	0/5
Dicofol[b]	2/5	0/5	0/5	0/5	0/5
Amitraz[c]	5/5	2/5	0/5	0/5	0/5
Propargite[b]	5/5	0/5	0/5	0/5	0/5
Hydramethylnon[d]	5/5	5/5	1/5	0/5	0/5
Cyhexatin[c]	5/5	5/5	0/5	0/5	0/5
Diafenthiuron[c]	5/5	5/5	0/5	0/5	0/5
DNOC[b]	0/5	0/5	0/5	0/5	0/5
Azocyclotin[c]	5/5	5/5	0/5	0/5	0/5
Pyridaben[d]	5/5	5/5	2/5	0/5	0/5
Chlorfenapyr[e]	5/5	5/5	5/5	3/5	0/5
Indoxacarb[d]	5/5	5/5	4/5	0/5	0/5
Carbaryl[c]	5/5	5/5	0/5	0/5	0/5
Spinosad[e]	5/5	5/5	5/5	5/5	4/5
Imidacloprid[d]	5/5	5/5	2/5	0/5	0/5
Diazinon[d]	5/5	5/5	3/5	0/5	0/5
Abamectin[e]	5/5	5/5	5/5	2/5	0/5
Permethrin[e]	5/5	5/5	5/5	5/5	4/5
Fipronil[e]	5/5	5/5	5/5	5/5	1/5

[a] Final concentrations are in the unit of part per billion (ppb).
[b] Highly inactive.
[c] Slightly active.
[d] Moderately active.
[e] Highly active.

Data Analysis. Bioassay data were analyzed using PoloPlus probit and logit analysis software (LeOra Software, Petaluma, CA). Control mortality was corrected using Abbott's formula. Chi-squared goodness-of-fit tests were performed, and LC_{50}/LC_{95} or LD_{50}/LD_{95} values were calculated using the PoloPlus program. Correlation analyses were performed using SigmaStat program (Systat Software, San Jose, CA).

Results and Discussion

To evaluate the accuracy and effectiveness of the HTS method using first-instar larvae of *Ae. aegypti*, we selectively chose 19 pesticides whose toxic properties against female *Ae. aegypti* had been determined earlier through topical application (Pridgeon et al. 2007). The 19 pesticides also represented different chemicals with a wide range of activities. The bioassay results are summarized in Table 2. As shown in Table 2, at a concentration of 500 parts per billion (ppb), bifenzate (a neuron inhibitor currently registered as a miticide with unknown mode of action), the least toxic pesticide against female adults of *Ae. aegypti*, showed no activity against first-instar larvae of *Ae. aegypti.* Our results also showed that dicofol (a registered miticide with unknown mode of action), the next least toxic pesticide against adult *Ae. aegypti*, caused no mortality in first-instar larvae at a concentration of 500 ppb (Table 2). Similarly, propargite, a registered miticide with low activity against adult *Ae. aegypti*, showed no activity against the first-instar larvae at a concentration of 500 ppb (Table 2). However, even at a low concentration of 2 ppb, three pesticides (spinosad, permethrin, and fipronil) that showed high activity against female *Ae. aegypti* were also very active against first-instar larvae (Table 2). By gradually decreasing the concentration of pesticide used in the HTS larval assay, we were able to group the 19 pesticides into four major groups: highly inactive; slightly active; moderately active; or highly active (Table 2).

To evaluate the correlation between our HTS larval assay and the topical application assay for adults, we determined the LC_{50} values of the 19 pesticides against first-instar larvae of *Ae. aegypti.* The results are summarized in Table 3. As shown in Table 3, permethrin and spinosad had the highest activity against first-instar larvae, with LC_{50} values of 0.28 and 0.39 ppb, respectively. This result was consistent with our initial HTS assay result, which showed that both spinosad and permethrin killed 80% of the first-instar larvae even at a low concentration of 0.5 ppb (Table 2). Based on LC_{50} values of the 19 pesticides, DNOC and bifenzate were the least active pesticides against

Table 3. Toxicities of 19 pesticides against first-instar larvae of *Ae. aegypti*

Pesticide name	LC_{50} (95% CI)[a]	LC_{95} (95% CI)[a]	Slope (SE)	χ^2
Dicofol	9.4×10^2 (8.8×10^2–9.9×10^2)	1.4×10^3 (1.2×10^3–1.7×10^3)	9.69 (1.73)	0.63
Bifenzate	2.8×10^3 (2.4×10^3–3.0×10^3)	4.3×10^3 (3.8×10^3–6.0×10^3)	8.79 (2.17)	0.60
Pyridaben	5.1×10^1 (4.0×10^1–5.7×10^1)	8.2×10^1 (7.3×10^1–1.1×10^2)	8.15 (2.10)	1.01
Indoxacarb	2.2×10^1 (1.5×10^1–2.6×10^1)	5.5×10^1 (4.4×10^1–9.3×10^1)	4.16 (1.01)	1.84
Amitraz	6.6×10^2 (4.9×10^2–7.7×10^2)	1.8×10^3 (1.3×10^3–4.1×10^3)	3.83 (0.99)	0.56
Hydramethylnon	4.0×10^1 (3.2×10^1–4.3×10^1)	6.3×10^1 (5.5×10^1–1.0×10^2)	8.22 (2.50)	0.75
DNOC	5.3×10^3 (4.3×10^3–5.8×10^3)	8.7×10^3 (7.4×10^3–1.8×10^4)	7.64 (2.40)	0.13
Chlorfenapyr	1.9×10^0 (1.7×10^0–2.0×10^0)	3.0×10^0 (2.6×10^0–4.0×10^0)	8.15 (1.63)	0.35
Propargite	7.8×10^2 (7.1×10^2–8.3×10^2)	1.1×10^3 (9.8×10^2–1.3×10^3)	12.4 (3.05)	0.03
Cyhexatin	2.7×10^2 (2.4×10^2–3.0×10^2)	4.2×10^2 (3.7×10^2–5.7×10^2)	9.01 (2.13)	0.15
Azocyclotin	2.0×10^2 (1.6×10^2–2.4×10^2)	7.8×10^2 (5.5×10^2–1.6×10^3)	2.84 (0.55)	0.87
Diafenthiuron	1.4×10^2 (1.1×10^2–1.5×10^2)	2.6×10^3 (2.2×10^2–4.0×10^2)	5.97 (1.45)	1.36
Abamectin	2.2×10^0 (1.9×10^0–2.4×10^0)	4.8×10^0 (4.0×10^0–6.9×10^0)	4.79 (0.84)	1.50
Spinosad	3.9×10^{-1} (3.6×10^{-1}–4.1×10^{-1})	6.3×10^{-1} (5.5×10^{-1}–7.9×10^{-1})	7.82 (1.33)	2.95
Imidacloprid	3.7×10^1 (2.9×10^1–4.5×10^1)	1.4×10^2 (8.7×10^1–5.3×10^2)	2.89 (0.75)	1.44
Permethrin	2.8×10^{-1} (2.5×10^{-1}–3.1×10^{-1})	5.5×10^{-1} (4.6×10^{-1}–7.3×10^{-1})	5.56 (0.72)	3.53
Fipronil	1.2×10^0 (0.9×10^0–1.6×10^0)	2.8×10^0 (2.0×10^0–9.1×10^0)	4.74 (0.76)	4.31
Carbaryl	4.2×10^2 (3.8×10^2–4.6×10^2)	9.2×10^2 (7.7×10^2–1.2×10^3)	4.78 (0.64)	2.95
Diazinon	2.7×10^1 (2.1×10^1–3.1×10^1)	7.0×10^1 (5.5×10^1–1.2×10^2)	3.94 (0.86)	1.66

[a] LC_{50} and LC_{95} values are in units of parts per billion (ppb).

the first-instar larvae, with LC_{50} values of 5,300 and 2,800 ppb, respectively (Table 3). These results were consistent with our findings from the initial HTS in which both DNOC and bifenzate did not kill any mosquitoes at concentration of 500 ppb (Table 2). Based on LC_{50} values of the 19 pesticides, dicofol and propargite were the next least active pesticides against first-instar larvae, with LC_{50} values of 940 and 780 ppb, respectively (Table 3). Our initial HTS also indicated that both dicofol and propargite were highly inactive against first-instar larvae, causing no mortality at a concentration of 500 ppb (Table 2). Based on LC_{50} values of the 19 pesticides, 5 pesticides (amitraz, carbaryl, cyhexatin, azocyclotin, and diafenthiuron) were slightly active against first-instar larvae, with LC_{50} values of 660, 420, 270, 200, and 140 ppb, respectively (Table 3). Our initial HTS also indicated these five pesticides were slightly active, causing some mortality at concentration of 500 ppb but no mortality at a concentration of 31.25 ppb (Table 2). Based on LC_{50} values, five pesticides (pyridaben, hydramethylnon, imidacloprid, diazinon, and indoxacarb) were moderately active against first-instar larvae, with LC_{50} values of 51, 40, 37, 27, and 22 ppb, respectively (Table 3). Our initial HTS also indicated that these five pesticides were moderately active, causing some mortality at a concentration of 31.25 ppb but no mortality at a concentration of 2 ppb (Table 2). Based on LC_{50} values, five pesticides (abamectin, chlorfenapyr, fipronil, spinosad, and permethrin) were highly active against first-instar larvae, with LC_{50} values of 2.2, 1.9, 1.2, 0.39, and 0.28 ppb, respectively (Table 3). Our initial HTS also indicated these five pesticides were highly active, causing mortality even at a low concentration of 2 ppb (Table 2). When the final concentration was decreased from 2 to 0.5 ppb, spinosad and permethrin continued to show high activity (80% mortality) against first-instar larvae in the initial HTS (Table 2), suggesting that spinosad and permethrin were the two most active pesticides against first-instar larvae. Taken together, our results suggest that the initial HTS method is suitable for evaluating the activities of chemicals as pesticides against *Ae. aegypti.*

To understand whether this HTS method could be used to predict a chemical's potency against adult mosquitoes, we compared the HTS larval assay results to the topical application assay results on adults using LD_{50}/LC_{50} value of permethrin as the standard. As shown in Table 4, bifenzate, the most inactive pesticide against adult *Ae. aegypti*, was also highly inactive against first-instar larvae, with LD_{50}/LC_{50} values that were 30,408- and 10,000-fold higher than that of permethrin, respectively (Table 4). Three other highly inactive pesticides (dicofol, amitraz, and propargite) in the adult bioassays were also highly inactive against the first-instar larvae, with LC_{50} values 3,357-, 2,357-, and 2,785-fold higher than that of permethrin, respectively (Table 4). However, fipronil, the most active pesticide against adult *Ae. aegypti*, was also highly active against first-instar larvae, with LC_{50} values only four-fold higher than that of permethrin (Table 4). Similarly, two other pesticides (spinosad and abamectin) that were highly active against female *Ae. aegypti* were also highly active against first-instar larvae, with LC_{50} values only one- and eight-fold higher than that of permethrin, respectively (Table 4). Our results also showed that three pesticides (cyhexatin, diafenthiuron, and azocyclotin) that were slightly active against adult *Ae. aegypti* were slightly active against first-instar larvae as well, with LC_{50} values >714-fold higher than that of permethrin (Table 4). Taken together, our results suggest that the HTS larval assay that we described here could be used initially to screen chemicals for their potency as pesticides for mosquito control. However, there were exceptions. For example, DNOC, a moderately active pesticide in the adult topical application assay, showed very low activity against first-instar larvae, with LD_{50}/LC_{50} values higher than that of permethrin for 510- and 18,929-fold, respectively (Table 3). A similar result was also observed for imidacloprid and carbaryl, both of which showed much lower activity against first-instar larvae than against the adult *Ae. aegypti.* The different activity data of the three pesticides (DNOC, imidacloprid, and carbaryl) in the larval and adult assays indicate that, in the real world, larval assay data and adult assay data do not always necessarily correlate with each other because many factors could affect a pesticide's performance. The physical/chemical parameters of a pesticide and its modes of action play important roles in its toxicity. Furthermore, critical

Table 4. Toxicity comparison of the 19 selected pesticides against *Ae. aegypti*

Pesticide no. and name	LD_{50} values[a]	LC_{50} values[b]	Toxicity (fold)[c]	
	Adult	Larvae	Adult	Larvae
Bifenzate	1.5×10^{0}	2.8×10^{3}	−30,408	−10,000
Dicofol	4.8×10^{-1}	9.4×10^{2}	−9,796	−3,357
Amitraz	4.1×10^{-1}	6.6×10^{2}	−8,367	−2,357
Propargite	2.4×10^{-1}	7.8×10^{2}	−4,898	−2,785
Hydramethylnon	2.0×10^{-1}	4.0×10^{1}	−4,082	−143
Cyhexatin	5.6×10^{-2}	2.7×10^{2}	−1,143	−964
Diafenthiuron	4.8×10^{-2}	1.4×10^{2}	−980	−500
DNOC	2.5×10^{-2}	5.3×10^{3}	−510	−18,929
Azocyclotin	8.8×10^{-3}	2.0×10^{2}	−180	−714
Pyridaben	3.0×10^{-3}	5.1×10^{1}	−61	−182
Chlorfenapyr	1.9×10^{-3}	1.9×10^{0}	−39	−7
Indoxacarb	1.5×10^{-3}	2.2×10^{1}	−31	−79
Carbaryl	9.5×10^{-4}	4.2×10^{2}	−19	−1,500
Spinosad	8.8×10^{-4}	3.9×10^{-1}	−18	−1
Imidacloprid	7.7×10^{-4}	3.7×10^{1}	−16	−132
Diazinon	6.7×10^{-4}	2.7×10^{1}	−14	−96
Abamectin	4.6×10^{-4}	2.2×10^{0}	−9	−8
Permethrin	4.9×10^{-5}	2.8×10^{-1}	1	1
Fipronil	4.6×10^{-7}	1.2×10^{0}	+107	−4

[a] LD_{50} values are in units of micrograms of pesticide per milligram of adult mosquito.

[b] LC_{50} values are in units of parts per billion against first-instar larvae.

[c] Toxicity is calculated according to the formula: Toxicity (fold) = (LD_{50} value of permethrin/LD_{50} value of pesticide) if the pesticide has higher toxicity than permethrin or Toxicity (fold) = (LD_{50} value of pesticide/LD_{50} value of permethrin) if the pesticide has lower toxicity than permethrin.

−, toxicity is lower than permethrin; +, toxicity is higher than permethrin.

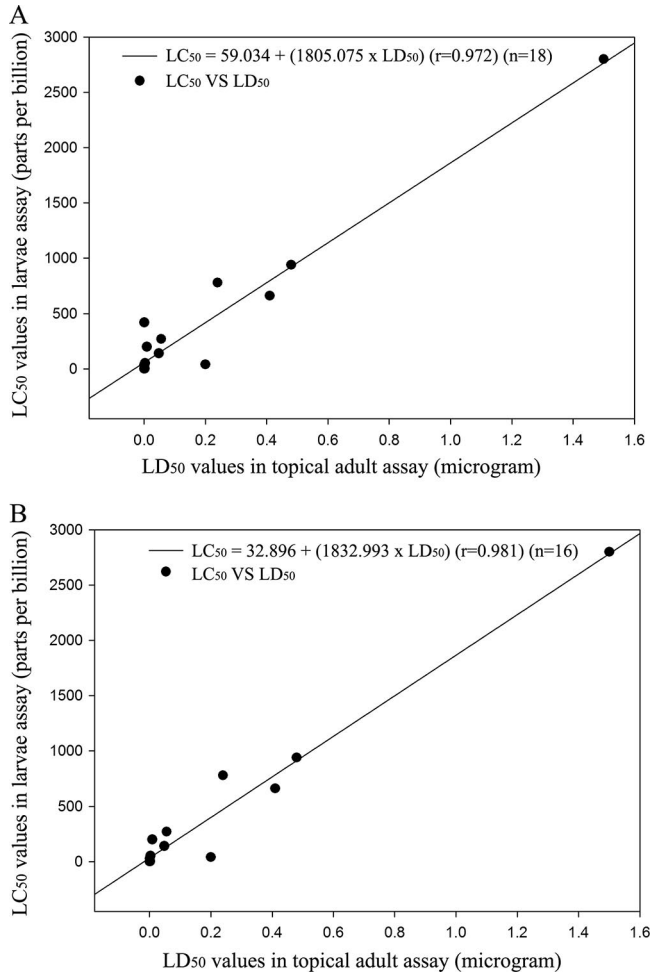

Fig. 1. Simple linear regression line between the LC_{50} values of pesticides in the larvae assay and the LD_{50} values in the topical adult assay. (A) Correlation of 18 pesticides. DNOC was the only pesticide that was excluded from the analysis. (B) Correlation of 16 pesticides. Three pesticides (DNOC, imidacloprid, and carbaryl) were excluded from the analysis.

target genes between different life stages could be differentially expressed, therefore affecting the performance of a pesticide against different life stages of a target pest.

To understand the exact correlation between the HTS larval assay and the adult topical application assay, Pearson product moment correlation coefficient, the most commonly used correlation coefficient, was calculated using SigmaStat statistic software. Using the LC_{50} values of the 19 pesticides in the larvae assay as one variable and the LD_{50} values in the topical adult assay as another variable, we found no significant correlation between the two, with correlation coefficients as low as 0.409 ($P = 0.0821$). However, when DNOC (a moderately active pesticide in the topical adult assay but with the worst larvicidal activity) was excluded in the correlation assay, we found a significant correlation between the LC_{50} values of the 18 pesticides in the larvae assay and the LD_{50} values in the topical adult assay, with correlation coefficients as high as 0.972 ($P < 0.05$; Fig. 1A). When all three pesticides (DNOC, imidacloprid, and carbaryl) were excluded in the correlation assay, significant correlation was found between the LC_{50} values and the LD_{50} values, with correlation coefficients as high as 0.981 ($P < 0.05$; Fig. 1B). Taken together, our results suggest that this HTS larval assay could be used to screen majority of the chemicals to predict their potency as adulticides for mosquito control.

To understand how fast and how efficient this HTS larval assay is, we used this assay to evaluate the activities of 130 test chemicals. The results are shown in Table 5. Based on 24-h mortality data, we were able to eliminate the majority of the test chemicals as highly inactive compounds (Table 5). By using three different concentrations (8, 2, and 0.5 ppm), we were able to sort the 130 test chemicals into four major groups: highly inactive, slightly active, moderately active, and highly active (Table 5). The 130 test chemicals included 111 plant chemicals and their derivatives, and the 19 pesticides (37–55) tested earlier as positive controls. Our results showed that the 19 pesticides

Table 5. Bioassay results of 130 test chemicals against the first-instar larvae of *Ae. aegypti* using the HTS method

No.	Dead/total			No.	Dead/total			No.	Dead/total			No.	Dead/total			No.	Dead/total		
	8[a]	2[a]	0.5[a]		8[a]	2[a]	0.5[a]		8[a]	2[a]	0.5[a]		8[a]	2[a]	0.5[a]		8[a]	2[a]	0.5[a]
1[b]	0/5	0/5	0/5	27[b]	0/5	0/5	0/5	53[e]	5/5	5/5	5/5	79[b]	0/5	0/5	0/5	105[b]	0/5	0/5	0/5
2[b]	0/5	0/5	0/5	28[c]	5/5	0/5	0/5	54[e]	5/5	5/5	3/5	80[b]	0/5	0/5	0/5	106[b]	0/5	0/5	0/5
3[b]	0/5	0/5	0/5	29[c]	1/5	0/5	0/5	55[c]	5/5	5/5	5/5	81[b]	0/5	0/5	0/5	107[c]	1/5	0/5	0/5
4[b]	0/5	0/5	0/5	30[d]	5/5	2/5	0/5	56[d]	5/5	2/5	0/5	82[b]	0/5	0/5	0/5	108[b]	0/5	0/5	0/5
5[b]	0/5	0/5	0/5	31[c]	3/5	1/5	0/5	57[d]	5/5	3/5	0/5	83[b]	0/5	0/5	0/5	109[b]	0/5	0/5	0/5
6[b]	0/5	0/5	0/5	32[c]	3/5	0/5	0/5	58[b]	0/5	0/5	0/5	84[b]	0/5	0/5	0/5	110[b]	0/5	0/5	0/5
7[b]	0/5	0/5	0/5	33[b]	0/5	0/5	0/5	59[c]	2/5	1/5	0/5	85[b]	0/5	0/5	0/5	111[c]	2/5	0/5	0/5
8[b]	0/5	0/5	0/5	34[b]	0/5	0/5	0/5	60[b]	0/5	0/5	0/5	86[b]	0/5	0/5	0/5	112[b]	0/5	0/5	0/5
9[b]	0/5	0/5	0/5	35[c]	2/5	0/5	0/5	61[b]	0/5	0/5	0/5	87[b]	0/5	0/5	0/5	113[b]	0/5	0/5	0/5
10[b]	0/5	0/5	0/5	36[b]	0/5	0/5	0/5	62[c]	2/5	0/5	0/5	88[b]	0/5	0/5	0/5	114[c]	1/5	0/5	0/5
11[b]	0/5	0/5	0/5	37[e]	5/5	5/5	2/5	63[c]	2/5	1/5	0/5	89[b]	0/5	0/5	0/5	115[b]	0/5	0/5	0/5
12[c]	1/5	1/5	0/5	38[d]	5/5	2/5	0/5	64[b]	0/5	0/5	0/5	90[b]	0/5	0/5	0/5	116[b]	0/5	0/5	0/5
13[b]	0/5	0/5	0/5	39[e]	5/5	5/5	5/5	65[b]	0/5	0/5	0/5	91[c]	1/5	0/5	0/5	117[c]	1/5	0/5	0/5
14[b]	0/5	0/5	0/5	40[e]	5/5	5/5	5/5	66[b]	0/5	0/5	0/5	92[b]	0/5	0/5	0/5	118[b]	0/5	0/5	0/5
15[b]	0/5	0/5	0/5	41[e]	5/5	5/5	2/5	67[b]	0/5	0/5	0/5	93[b]	0/5	0/5	0/5	119[b]	0/5	0/5	0/5
16[c]	5/5	0/5	0/5	42[e]	5/5	5/5	5/5	68[b]	0/5	0/5	0/5	94[c]	3/5	0/5	0/5	120[c]	3/5	0/5	0/5
17[d]	5/5	5/5	0/5	43[c]	5/5	0/5	0/5	69[b]	0/5	0/5	0/5	95[b]	0/5	0/5	0/5	121[c]	3/5	0/5	0/5
18[c]	5/5	2/5	0/5	44[e]	5/5	5/5	5/5	70[d]	5/5	2/5	0/5	96[b]	0/5	0/5	0/5	122[b]	0/5	0/5	0/5
19[c]	5/5	2/5	0/5	45[d]	5/5	5/5	0/5	71[e]	5/5	4/5	2/5	97[b]	0/5	0/5	0/5	123[b]	0/5	0/5	0/5
20[b]	0/5	0/5	0/5	46[e]	5/5	5/5	5/5	72[e]	5/5	5/5	2/5	98[c]	1/5	0/5	0/5	124[c]	4/5	0/5	0/5
21[b]	0/5	0/5	0/5	47[e]	5/5	5/5	5/5	73[e]	4/5	4/5	3/5	99[b]	0/5	0/5	0/5	125[c]	5/5	0/5	0/5
22[e]	5/5	5/5	2/5	48[e]	5/5	5/5	5/5	74[d]	5/5	3/5	0/5	100[b]	0/5	0/5	0/5	126[c]	2/5	0/5	0/5
23[b]	0/5	0/5	0/5	49[e]	5/5	5/5	5/5	75[c]	3/5	0/5	0/5	101[b]	0/5	0/5	0/5	127[b]	0/5	0/5	0/5
24[b]	0/5	0/5	0/5	50[e]	5/5	5/5	5/5	76[b]	0/5	0/5	0/5	102[b]	0/5	0/5	0/5	128[b]	0/5	0/5	0/5
25[c]	2/5	0/5	0/5	51[e]	5/5	5/5	5/5	77[e]	5/5	4/5	1/5	103[b]	0/5	0/5	0/5	129[e]	5/5	5/5	4/5
26[b]	0/5	0/5	0/5	52[e]	5/5	5/5	5/5	78[b]	0/5	0/5	0/5	104[b]	0/5	0/5	0/5	130[e]	5/5	4/5	1/5

[a] Final concentrations are in the unit of part per million (ppm).
[b] Highly inactive.
[c] Slightly active.
[d] Moderately active.
[e] Highly active.

were either slightly active (43), moderately active (38 and 45), or highly active in the initial HTS (Table 5). Our results also showed that the majority of the plant chemicals and their derivatives were either highly inactive (72 of 111; Table 5, footnote b) or slightly active (26 of 111; Table 5, footnote c) against first-instar larvae (Table 5). However, a small portion (13 of 111) of the test chemicals were found to be either moderately active (6 of 111; Table 5, footnote d) or highly active (7 of 111; Table 5, footnote e) against first-instar larvae. These 13 chemicals will be used for further structure-activity analysis so that highly active compounds might be developed for use in mosquito control.

There are several advantages to using this HTS method to evaluate the activity of test chemical. First, this method is very fast. We were able to get the primary screen result of 130 test chemicals within 3 d (day 1, hatch egg; day 2, sort larvae into 24 well plates and add chemicals into the well; day 3, score mortality). We are able to sort larvae into 200 wells (five larvae per well) within 1 h per person, so we are able to perform the assay in 600 wells/d or 1,800 wells/wk. Second, this method is rather easy in that it uses freshly hatched (1 d old) *Ae. aegypti* larvae, which eliminates the need to sort mosquitoes of a specific age. For example, the commonly used WHO standard larvae assay (WHO 1981) involves rearing and sorting out fourth-instar larvae. Third, this method only requires a supply of very small amount of test chemical in that it only uses 10 μl of diluted test chemicals in a final volume of only 1 ml, which reduces the amount of test chemicals significantly. If a test chemical does not show activity at a final concentration of 8 ppm (8 μg of test chemical in 1 ml of water), that chemical is not considered to be suitable as a leading candidate compound for structure modification. We only need 8 μg of test material to produce a preliminary result at a final concentration of 8 ppm. If we use other mosquito larvae assay methods that have final volumes of 250 and 100 ml, respectively, as used by Selvi et al. (2007) or Paul et al. (2006), we need 2,000 and 800 μg of test chemicals, respectively, to get a final concentration of 8 ppm. Therefore, this HTS assay will be more suitable when only limited amount of chemicals are available. Fourth, this method is relatively inexpensive because it does not involve rearing mosquitoes to a specific age, thus reducing the costs and labor involved in rearing mosquitoes for assay. The small amount of test material also reduces the costs and labor associated with the generation of large amounts of test materials. Fifth, our HTS method is space friendly in that our HTS assay uses 24-well plates, which can be stacked together so that hundreds and thousands of assays could be performed on 1 d, whereas other larvae assay systems have larger space requirements to screen large numbers of compounds.

In summary, a fast, easy, inexpensive, and space friendly HTS method using the first-instar larvae of *Ae. aegypti* was developed and evaluated in our labora-

tory. LC_{50} values of a representative group of compounds were determined using this HTS method and compared with LD_{50} values determined by topical application against adult *Ae. aegypti.* Our results showed that this method is highly efficient and has the potential for screening thousands of chemicals quickly to identify new chemicals that may be effective for controlling mosquitoes.

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